UNPUBLISHED PRELIMINARY DATA

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QUARTERLY PROGRESS REPORT NO. 9

TO NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

RADIOISOTOPIC BIOCHEMICAL PROBE FOR EXTRATERRESTRIAL LIFE

CONTRACT NO. NASr-10

June 25, 1963

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I. SUMMARY

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Garden and field soil inocula have consistently responded well in M8 medium containing various radioactive substrates. Four bacteria and two mycelial fungi isolated from soils responded positively when tested in M8 medium with labeled formate and glucose. Bacillus subtilis var. globigii has been added as a test organism and responds well when vegetative cells are used for inoculum. Spores alone were negative and require further investigation. Rhodospirillum rubrum was tested photosynthetically and responded well, but at a lower level than when tested in the dark.

A variety of radioactive substrates, including DL-malic acid-3-C^{14}; glycine-1-C^{14}; urea-C^{14}, and nitrilotriacetic-C^{14} acid were tested. The most significant response from soil inocula resulted when glycine was used in combination with formate and glucose. Malic acid and urea were both utilized, but not as well as the glycine. Nitrilotriacetic acid was not satisfactory at the levels tested.

It was also determined that sodium formate at a specific activity of 25 mc/mM results in more detectable $C^{14}O_2$ than the sodium formate previously used which had a specific activity of 5 mc/mM. The most advantageous concentration of the higher specific activity formate appears to be 0.2 mM, or 5 uc/ml.

The feasibility of using Gulliver as a light-dark chamber to elicit responses from photosynthetic organisms has been further substantiated.

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Efforts to establish sensitivity values have resulted in a measurable response within 30 minutes from as few as 118 cells of a bacterium isolated from soil. Other cells, ranging from approximately 30,000 to 230,000 per test, responded equally as well as the soil isolate. One bacterium tested, required between three million and seven million cells for a positive 30 minute response.

All field tests (3) resulted in positive responses.

II. BIOLOGICAL INVESTIGATION

The biological investigation conducted during the past quarter included:

(1) a study of responses from soils and soil isolates,

(2) a study of responses from old and new pure cultures of test organisms,

(3) examination of new substrates,

(4) investigation of increased specific activity levels and increased concentrations of sodium formate-C^{14},

(5) development of a means to detect photosynthesis, and

(6) field tests.

A. RESPONSES OF TEST MICROORGANISMS

1. Soil Isolates

Throughout the entire biological program, soils have been an important source of microorganisms. The pure cultures of selected laboratory test organisms yield essential data, but complete evaluation of the basal medium must be augmented by mixed population studies. Field, garden, and desert soils have been utilized extensively in laboratory as well as field investigations. (Results reported in Second Annual Progress Report).

Soil responses in the basal medium, M8, have been generally high and satisfactory. Results of soils tested during this quarter are

reported under the sections "New Substrates" and "Effects of Increased Specific Activity." To examine some of the organisms in the field and garden soils most commonly utilized as inocula, the soils were plated for isolation of individual microbial genera. Approximately 125 mg of each soil were suspended and shaken in five ml of sterile water. One loopful of the suspension was streaked on Difco Sabouraud Agar containing Chloromycetin and another on Difco Nutrient Agar containing Fungizone. Following incubation at room temperature, the plates were examined for the most frequently occurring colonies. Six were selected and subcultured until pure cultures were obtained. Some general characteristics of the isolates are shown in Table 1.

Concurrently, total colony counts were determined on the two soils which were plated. The field soil yielded 88,500 colonies per 100 mg when plated on Nutrient Agar with Fungizone and 230 on Sabouraud Agar with Chloromycetin. The garden soil yielded 112,500 colonies per 100 mg on Nutrient Agar and 365 colonies on Sabouraud Agar.

Experiments were carried out using each of the soil isolates as inoculum. One ml of M8 medium, containing 5 μc formate (1×10^{-3} M) and 1 μc glucose (3×10^{-4} M) per ml was seeded with 0.1 - 0.6 ml of diluted or undiluted inoculum (amount used depended upon the growth of the stock culture). The cultures were incubated in chambers with the resulting $C^{14}O_2$ monitored by the automatic recording

TABLE 1

SOME GENERAL CHARACTERISTICS OF

SOIL ISOLATES

Isolate	Soil source	Type	Gram Stain	Characteristics
A	Field and Garden	Bacterium	Gram positive bacillus	White, lacy colonies growing in swirl arrangements
B	Field	Bacterium	"	Small, circular, opaque colonies
C	Garden	Bacterium	"	Pale yellow, circular colonies
D	Field	Bacterium	"	Dull, white colonies producing brown pigment in the medium
E	Field and Garden	Fungus		Green changing to dark brown mycelial growth, Penicillium-like
F	Field and Garden			White mycelial growth

unit. Each determination included a corresponding plate count. Results are shown in Table 2.

A comparative study of the soil isolates was also performed using planchets. In previous mixed soil populations, DL-sodium lactate-1-C^{14} incorporated as a substrate in the M8 medium, yielded excellent responses. Therefore, in this study, the sodium lactate was used as the sole source of labeled carbon. The experiment was carried out as follows: 0.5 ml of M8 medium containing 6.65 μc/ml (1.33 mM) of lactate-1-C^{14} was inoculated at room temperature for two hours and $C^{14}O_2$ collected on pads containing saturated $Ba(OH)_2$ for 15 minutes. $C^{14}O_2$ collections were made again following an additional two hours of incubation.

When tested in planchets, only three of the six isolates responded. However, when tested in the automatic system using more media and larger inocula, positive results were obtained from each isolate (Table 2). Soil isolate E, a fungus, is not listed in Table 2 because it failed to grow on plates, thereby making cell numbers unavailable. Nevertheless, 0.2 ml of a 24 hour broth culture evolved 50 to 80 CPM above background within 30 minutes. The positive responses from soil isolates E and F are particularly significant because they represent the first tests with mycelial fungi. Admittedly, plate counts with mycelial fungi are difficult to make, but the cultures (F) grew initially as discrete colonies and enabled an approximate determination to be made. The response from the 58,000 - 87,000 cells was reasonably good within the first few hours and quite good at four hours.

TABLE 2

RESPONSE TO M8 MEDIUM

Sample	Cells Per Test	C-14 Substrate	Radioactivity-CPM Above Sterile Control — Time in Hours 0.5	1	2	4	8
Soil Isolate A Bacterium	66,000	1	2,145	3,390	6,350	12,970	19,900
Soil Isolate B	29,800	1	30	80	375	2,235	6,790
Bacterium	59,600	1	165	240	735	4,335	8,790
Soil Isolate C	3,700,000	1	0	0	0	120	490
Bacterium	7,400,000	1	65	65	165	420	890
Soil Isolate D	118	1	75	345	590	1,290	3,360
Bacterium	177	1	165	465	585	1,590	5,160
Soil Isolate F	58,400	1	50	120	270	1,120	4,590
Fungus	87,600	1	110	150	330	1,350	4,590
Rhodospirillum rubrum (photo-synthetic growth)	220,000	2	120	180	360	940	1,545
Rhodospirillum rubrum (non-photosynthetic)	181,000	2	1,040	1,615	2,485	4,430	8,270
Bacillus subtilis var. globigii	70,000	1	65	135	405	2,060	11,800
Pseudomonas fluorescens	228,000	2	130	135	315	450	800
Garden Soil	112,865	3	2,260	4,030	8,890	16,345	20,520
Garden Soil	112,865	4	4,342	7,170	13,010	18,893	22,180
Field Soil	88,730	4	585	1,225	2,340	5,430	10,625
Field Soil	88,730	3	560	1,015	2,405	5,019	9,450

1. Formate (1.0 mM) + Glucose (0.33 mM)
2. Formate (0.66 mM) + Glucose (0.22 mM) + Lactate (0.44 mM)
3. Formate (0.66 mM) + Glucose (0.22 mM) + Malic (0.44 mM)
4. Formate (0.66 mM) + Glucose (0.22 mM) + Glycine (0.44 mM)

Because of the inherent selectivity of any plating method for isolating organisms from soil, the six isolates obtained from the two soils represent only a fraction of the organisms present. The positive responses from soil may be the result of the combined metabolism of the heterogeneous population or the relatively high activity of only a few of the organisms. The limited sampling reported above shows that at least four of the six organisms actively evolved $C^{14}O_2$ from the M8 medium and the other two responded positively but at a more reduced level.

To attempt to completely isolate and test, as pure cultures, every member of each test soil is a task too extensive to be undertaken. Therefore, in order to retain at least some degree of the natural factors which are contributed by soils to many organisms, pure cultures will be transferred through sterilized soils for future testing. They will also be tested from culture media. In this way, it is hoped that the program can take advantage of a wide range of pure cultures and simultaneously determine the responses from those cultures in soils.

2. Known Cultures

The use of identified cultures as test organisms has been extended with the addition of Bacillus subtilis var. globigii to the test collection. This organisms has the characteristic of producing extremely resistant spores. Vegetative cells and spore suspensions were each used as inoculum for various determinations. The spore suspension was prepared by pasteurizing a culture of vegetative cells. All determinations were made using the automated system.

The vegetative cells were tested in basal M8 medium containing formate and glucose, formate-glucose-lactate, or lactate alone as the radioactive substrates which were used at a total concentration of 1.33 mM and in the proportions described under the section "New Substrates" of this report. A positive response was obtained in every determination, but the response to a particular substrate or combination of substrates was variable. At one time the combination of formate and glucose appeared to be the best. At another time, the combination of formate-glucose-lactate appeared better than any other, and at still another time, the organisms used lactate alone as well as any of the combinations. The variations might be a reflection of size of inoculum or proportion of spores to vegetative cells since there undoubtedly was a mixture. It is certain, however, that a relatively good response is obtained.

The use of a spore suspension to inoculate combinations of radioactive substrates in M8 media yielded less satisfactory results. Again the combinations were used as described under "New Substrates". Combinations of formate-glucose, formate-glucose-lactate, formate-glucose-glycine, as well as lactate and glycine used singly were all tested in M8 medium. Response from the spores was essentially negative in every case.

This suggests that one of several approaches must be taken to elicit good responses from spore inocula. One approach is to find other radioactive substrates which will penetrate the spore and which can be used more readily than the limited number tried to date. A second approach is to add a constituent which will promote germination rapidly since good, positive responses are obtained from vegetative cells. A third approach would utilize a combination of the first two.

Pseudomonas fluorescens was used as a test organism in M8 basal medium containing the formate-glucose, formate-glucose-lactate, combinations or lactate alone as described in the section "New Substrates". Positive responses were obtained in all tests, but the formate-glucose combination appeared to have advantages over the other substrate combinations.

Rhodospirillum rubrum was tested in the M8 medium with glycine-C^{14} added as described under "New Substrates." The response to the combination of formate-glucose-glycine was the best. It was also tested in formate-glucose-lactate photosynthetically, utilizing

the chambers used for the Chlorella experiments. A positive response was obtained, and when compared to an experiment carried out with essentially the same number of cells as inoculum, the absolute response observed photosynthetically was less than when the cells were tested in the dark (Table 2). This result is in complete agreement with the Chlorella photosynthetic experiments.

3. Sensitivity

Cell numbers of the inocula used in the determinations of the responses from known cultures, soil isolates, and the soils themselves were determined by plate count. The activity above the sterile controls is shown in Table 2. Using the greatest response from the fewest organisms in the shortest time as a measure of sensitivity it can be seen that considerable variation exists. Soil Isolate D appears to be quite active with as few as 118 cells yielding a measurable response at the initial determination of 30 minutes. Because of this extremely high response a recheck is being done and will be reported in the next progress report. It is obvious that if measurements had been made prior to the 30 minute period on the Soil Isolate A, Rhodospirillum rubrum grown in the dark, and the soils, positive responses would have been obtained. It is probable that some of the other test organisms would have been detectable earlier. The least responsive organism was Soil Isolate C, a bacterium.

The sensitivity, as defined, is dependent upon the organism being tested. When the size of inoculum used in most of the

determinations (Table 2) is considered, relative to the greater numbers of cells normally used for inocula in routine laboratory procedures and commonly found in soils, the method is quite sensitive and does detect the metabolism of small numbers of cells. Furthermore, there is every reason to believe that with continued improvement in the medium, soil sampling procedure, and detection techniques, Gulliver will become even more sensitive.

B. NEW SUBSTRATES

Investigations of new labeled substrates for their incorporation into the basal medium has continued. Determinations were carried out using DL-malic acid-3-C^{14}, specific activity - 6.8 mc/mM; glycine-1-C^{14}, specific activity - 4.42 mc/mM; urea-C^{14}, specific activity 2.05 mc/mM; and nitrilotriacetic-C^{14} acid, specific activity - 0.02 mc/mM. Since this is a continuation study, all experimental factors were kept at the previous levels. These were a radioactive ratio of 5 µc formate/1 µc glucose; a molar ratio of 3 formate/1 glucose (specific activity - formate 5.0 mc/mM, glucose 3.0 mc/mM); and a total concentration of 1.33 mM. Determinations were made using 1.0 mM of formate and 0.33 mM of glucose (total 1.33 mM) as the standard; 0.66 mM formate, 0.22 mM glucose, 0.44 mM of the new substrate (total 1.33 mM) as the combination investigated or 1.33 mM of the new substrate alone. Except for the use of Escherichia coli to test the nitrilotriacetic acid, 100 mg of either field or garden soil were cultured in 0.2 ml of the desired labeled M8, monitored, and recorded on the $C^{14}O_2$ automatic monitoring unit.

The most significant response obtained was with the glycine (Figure 1). Not only did the formate-glucose-glycine combination elicit a response equal to, or better than, the basic formate-glucose substrates, but it resulted in a good response when used as the only C-14 substrate. Further investigations of this substrate for possible inclusion into the basal medium are being continued.

The urea and malic acid substrates produced unsatisfactory results. Although each was utilized by the organisms when added alone, and in combination with formate and glucose, the responses obtained were not pronounced enough to warrant further investigation. The nitrilotriacetic acid,a chelating agent used in another experimental program, yielded poor responses in all combinations. It is of interest because of its nitrogen and acetic acid components, but it is possible that the low specific activity (220 times less than that of the glycine) was insufficient for a good response. It is also possible that the compound was not readily utilized.

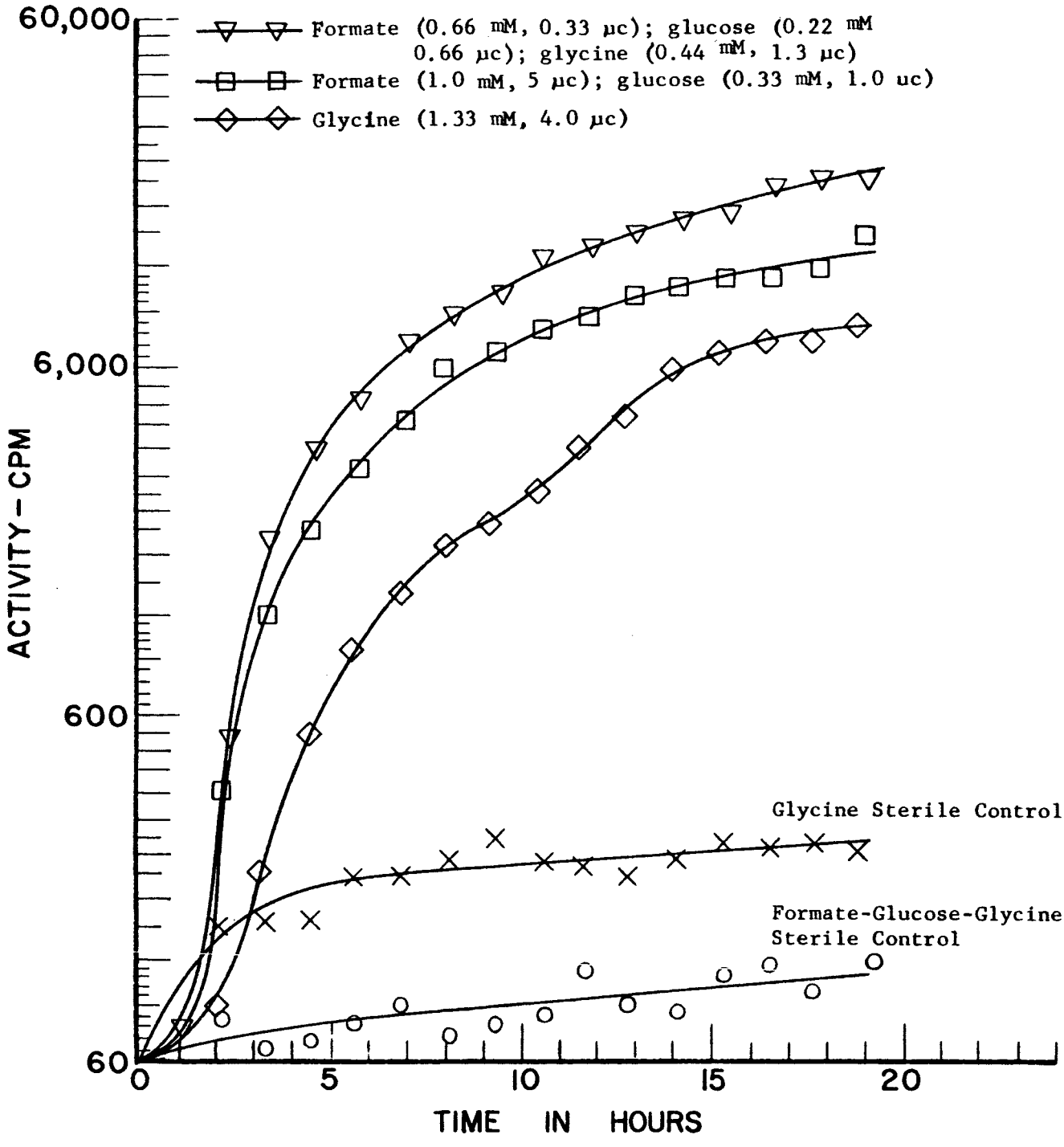
Efforts to determine new substrates for incorporation into the basal medium are continuing.

C. EFFECTS OF INCREASED SPECIFIC ACTIVITY

During the past year, detailed investigations on formate-C^{14} and glucose-C^{14} indicated that the most satisfactory response was obtained by using a 3/1 molar ratio and a 5/1 radioactive ratio (5 μc formate to 1 μc glucose per ml test medium). The formate had a specific activity of 5 mc/mM, the glucose 3 mc/mM. The continuation

FIGURE 1

$C^{14}O_2$ FROM 100 MG OF SOIL IN M8 MEDIUM WITH FORMATE-GLUCOSE, FORMATE-GLUCOSE-GLYCINE, OR GLYCINE ALONE AS RADIOACTIVE SUBSTRATES



of this study has led to the investigation of labeled substrates with a higher specific activity. Due to the relative importance of formate, this substrate was considered first.

Formate having a specific activity of 25 mc/mM was compared with formate having a specific activity of 5 mc/mM on a molar and radioactivity basis. Since the combination of formate and glucose has been the basic radioisotopic substrate standard, glucose was consistently incorporated at a level of 3×10^{-4} M (1 μc/ml). The three combinations tested were:

(1) Formate specific activity - 25 mc/mM
Radioactivity - 25 μc formate/1 uc glucose per ml
Total activity - 26 μc/ml
Molarity - 1.0 μmole formate/0.33 μmole glucose per ml

(2) Formate specific activity - 25 mc/mM
Radioactivity - 5 μc formate/1 uc per ml
Total activity - 6 μc/ml
Molarity - 0.2 μmole formate/0.33 μmole glucose per ml

(3) Formate specific activity - 5 mc/mM
Radioactivity - 5 μc/formate/1 μc glucose per ml
Total activity - 6 μc/ml
Molarity - 1.0 μmole formate/0.33 μmole glucose per ml

The automated $C^{14}O_2$ monitoring unit was used. Two-tenths ml of the labeled M8 medium was inoculated with 100 mg of either field

or garden soil, or when a pure culture of Escherichia coli was used, 0.5 ml of medium was seeded with 0.2 ml of inoculum. The necessary sterile controls were incorporated throughout.

The results shown in Figure 2 can be summarized as follows:

1. The formate of higher activity yielded the best response when used at a concentration of 1.0 mM (25 μc/ml).
2. The next highest response resulted from the higher activity formate at a concentration of 0.2 mM (5 μc/ml).
3. The lower activity formate at a concentration of 1.0 mM (5 μc/ml) elicited the lowest response.

Because it is essential to hold nonmetabolic activity to a minimum and to keep the medium constituents from becoming inhibitory, it appears to be advantageous to use the higher specific activity formate at the lower of the two concentrations tested. Further investigations will be carried out to determine the possibility of utilizing a lower molarity of formate at the 25 mc/mM level or, perhaps, even higher specific activity levels.

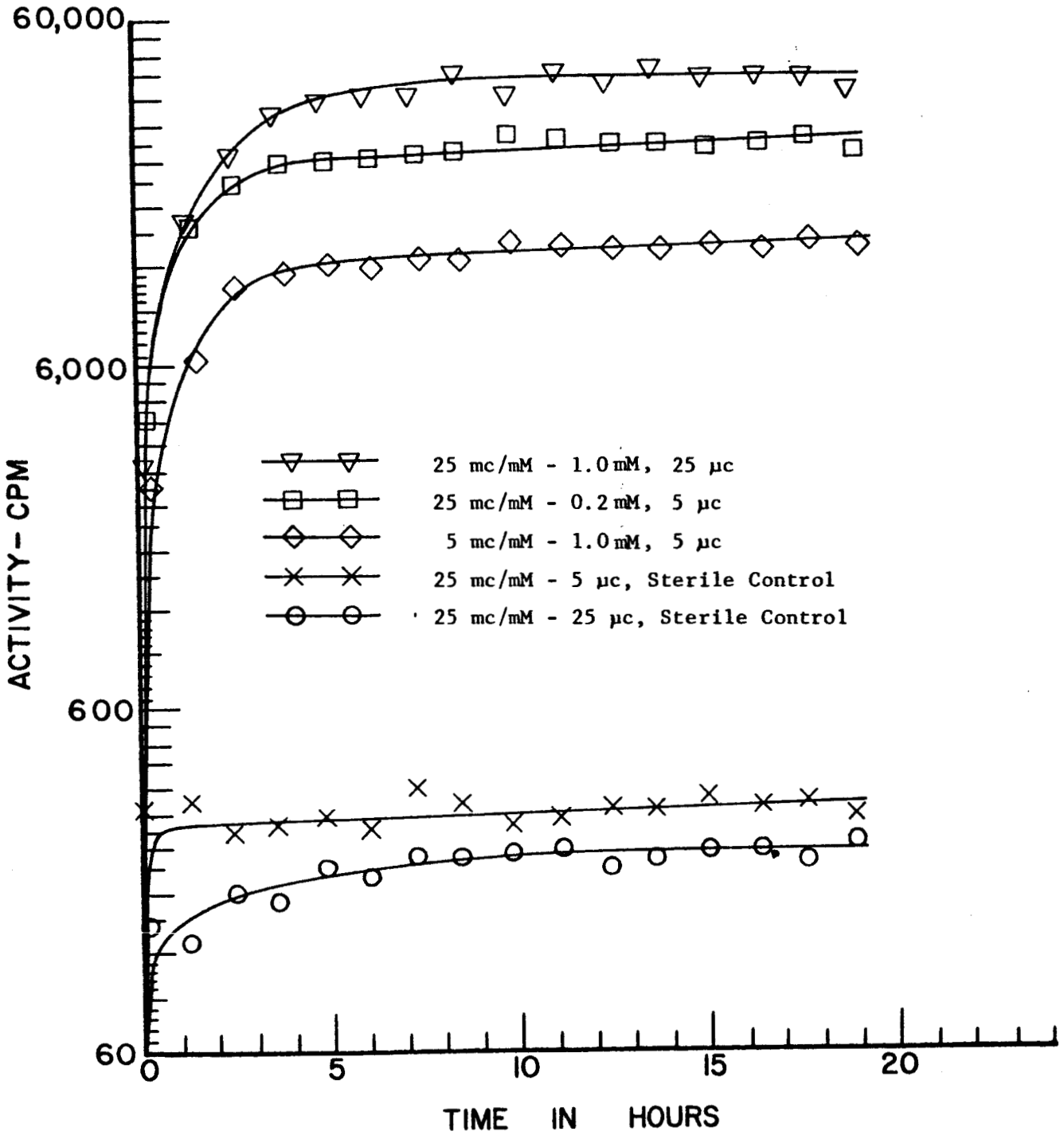
Comparative studies are planned for the other major radioactive substrates.

D. MEDIUM DEVELOPMENT

Investigation of the unlabeled components of the basal medium has continued. The influence of one substrate upon the utilization of another is an established fact. For example, some

FIGURE 2

EFFECT OF SPECIFIC ACTIVITY OF SODIUM FORMATE-C^{14} ON RESPONSE FROM SOIL INOCULA



carbon sources are better utilized in the presence of some nitrogen sources than they are in the presence of others. The utilization of nitrogen sources is also influenced by the carbon source in the medium. Recently, Levin (Reducing Secondary Effluent Phosphorus Concentration, Progress Report #1, District of Columbia and Public Health Service, April, 1963) has shown that sodium succinate added to glucose in a 3/1 molar ratio enhances orthophosphate uptake by microorganisms in sewage. This suggested the possibility that sodium succinate in the basal medium might influence the utilization, and subsequent CO_2 evolution of the radioactive substrates. To investigate this, non-radioactive sodium succinate was added to the basal M8 medium in a 3/1 molar ratio with the glucose-C^{14}. Cultures of Saccharomyces cerevisiae, Escherichia coli and Soil Isolate C were used singly for inoculum, and responses were measured on the automatic monitoring unit. Detectable $C^{14}O_2$ was not increased in any of the determinations in the presence of nonradioactive sodium succinate Nevertheless, it is likely that the succinate was used and that CO_2 was evolved from its utilization. Therefore, experiments are planned in which succinic-1-C^{14} acid will be tested as a supplementary substrate.

E. PHOTOSYNTHESIS

Investigations reported in a previous Progress Report indicate that when D-glucose-C^{14} or DL-sodium lactate-1-C^{14} are added to a basic algal culturing medium containing urea as a nitrogen source, a marked response in detectable $C^{14}O_2$ occurs during light-dark culturing.

The planchet method described in the Second Annual Report was used to determine the effect of several light-dark cycles upon the evolution of $C^{14}O_2$ from Chlorella pyrenoidosa cultured on urea agar. In the first of these experiments, the urea agar was supplemented with DL-sodium lactate-1-C^{14} in a concentration of 1×10^{-3} M and an activity of 5 μc/ml. As before, two sets of experimental chambers were used. In one set, light was admitted, and in the other set, light was excluded. The chambers were maintained in this manner for one hour and thirty-five minutes at which time they were reversed, the light phase being changed to the dark, and the dark to the light. This procedure was repeated every hour thereafter for a total of five cycles. A light control chamber, maintained in the light continually, and a dark control chamber maintained in the dark continually, were run concurrently. All chambers were inoculated with approximately 5×10^6 cells. The results (Figure 3) show a definite response to each light change with the radioactivity levels fluctuating between the appropriate controls.

The concentration of lactate apparently was adequate, but a further examination of the effect of increased substrate concentration upon the evolution of $C^{14}O_2$ by the algae seemed warranted. A 2×10^{-3} M (10 μc/ml activity) concentration of DL-sodium lactate-1-C^{14} was added to the basic urea agar, and each chamber was inoculated with about 5×10^6 cells. The same experimental procedure described above was utilized. As indicated in Figure 4, the cultures exhibit a

FIGURE 3

$C^{14}O_2$ EVOLVED BY CHLORELLA PYRENOIDOSA

IN RESPONSE TO LIGHT AND DARK GROWTH CYCLES

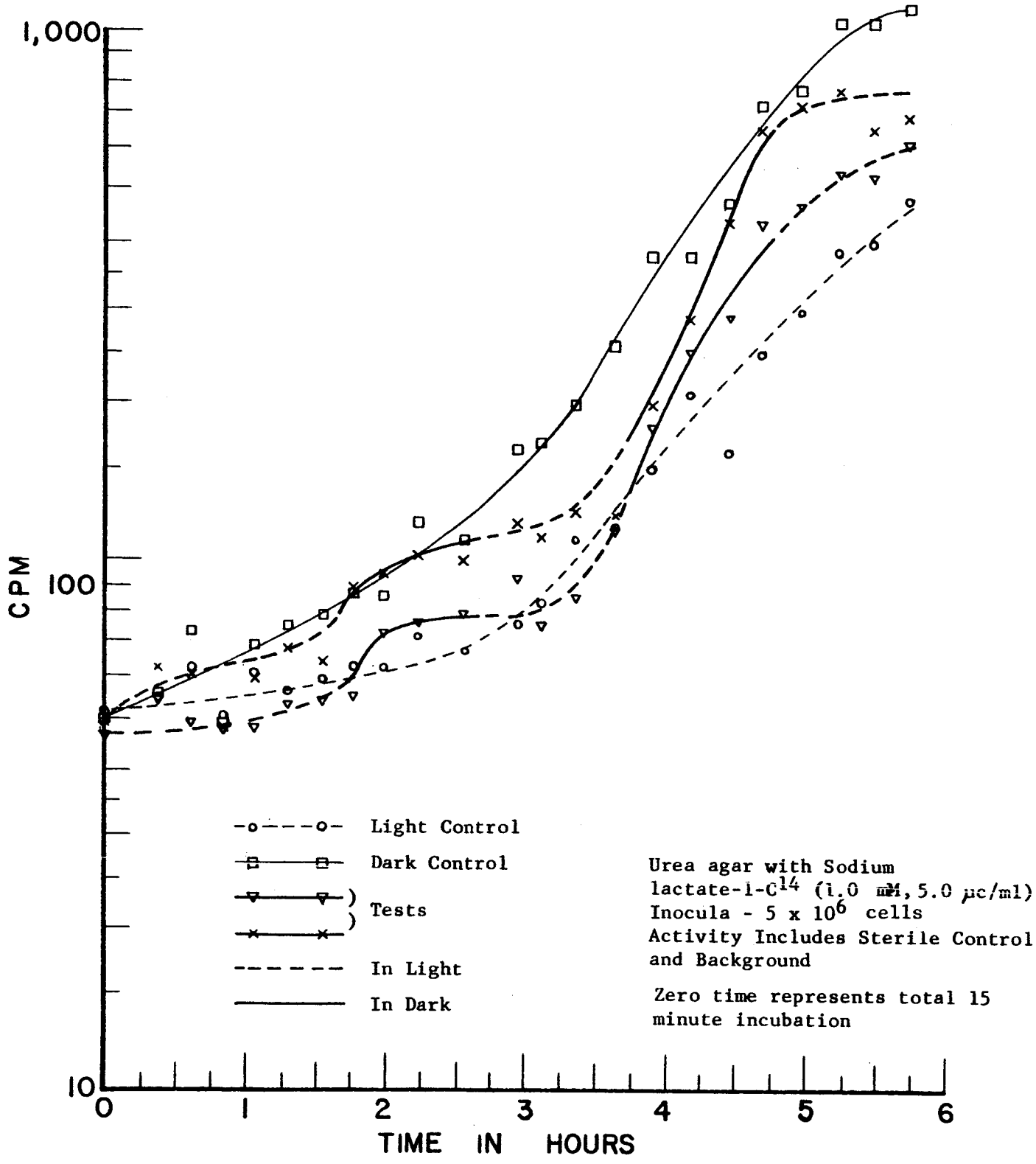
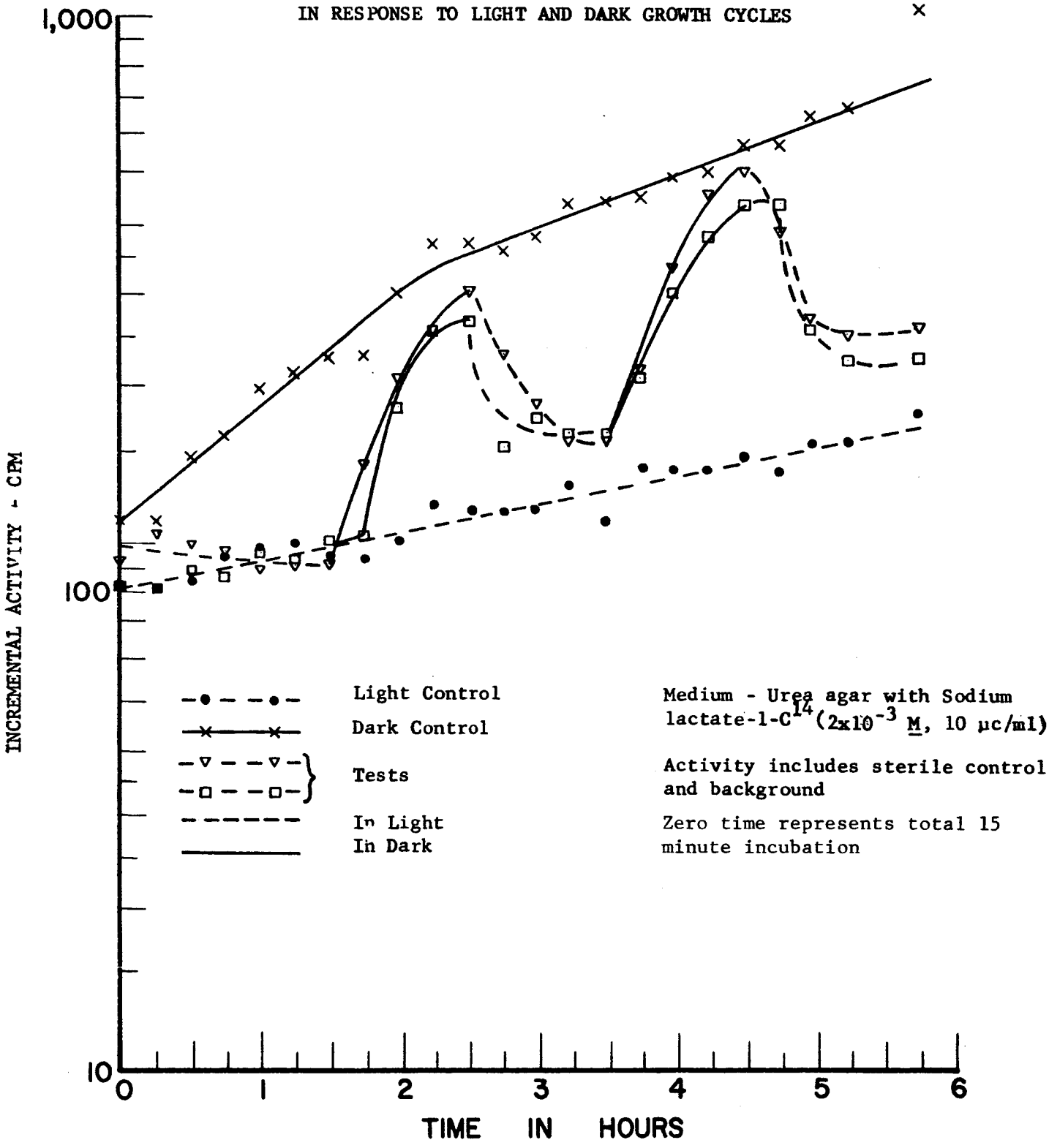


FIGURE 4

$C^{14}O_2$ EVOLVED BY CHLORELLA PYRENOIDOSA
IN RESPONSE TO LIGHT AND DARK GROWTH CYCLES



more marked response to light change when the concentration of substrate is doubled. Again, the activity of the inoculated chambers falls within the control values. By starting the inoculated chambers in the light, the initial response to light change is quite pronounced.

Thus far, in the effort to establish the feasibility of using Gulliver as a photosynthetic experiment, a basic salts medium with urea as a nitrogen source has been used exclusively. However, urea may not be the best nitrogen source for culturing the majority of algal species, especially blue-green algae. Experiments were conducted to determine the effect of a different nitrogen source upon the evolution of $C^{14}O_2$ during light-dark culturing. KNO_3 at a level of 0.34 g/ml was substituted for urea in the basic medium, and 2×10^{-3} M DL-sodium lactate-1-C^{14} was used as the radioactive substrate. The Chlorella pyrenoidosa used for inoculation was grown in KNO_3 medium prior to the experiment. Response to light change was the same as obtained in urea medium, but the absolute levels of evolved $C^{14}O_2$ were much lower. This may be a result of the high pH (8.5) of the KNO_3 culture compared to the pH of 6.5 of urea cultures.

It is virtually certain that if life exists on Mars, there will be at least one photosynthetic species. This makes it important to be able to determine their existence, and to be able to demonstrate

a photosynthetic metabolism. The experiments reported above, further establish the feasibility of using the basic approach of Gulliver to demonstrate the presence of photosynthesizing organisms by their response to light-dark cycles. Efforts are continuing toward refinement of the entire photosynthetic aspect of Gulliver.

F. FIELD TESTS

Although field tests have been conducted previously, a more extensive program was initiated in order to evaluate and, if necessary, to modify, the instrument and testing procedures. It also provides an opportunity to evaluate laboratory-developed media under field conditions.

Currently, two instruments are sterilized and tested simultaneously. Each contains an ampule of medium and an ampule of antimetabolite. They are programmed to perform the same operations, although one sequence is completed before the second is begun. This results in approximately 10 minutes difference between the two instruments. Firing of the antimetabolite is not programmed with the other functions, thereby permitting selection of the instrument and time of introduction of the antimetabolite on the basis of response.

The results of the field tests are presented in an abbreviated form to permit a complete presentation. Positive responses have been obtained from the three field tests conducted this quarter. The absolute values of $C^{14}O_2$ detected vary from test to test and were low to moderate in the first two. Nevertheless, a definite difference is obvious between the uninhibited and inhibited test chambers. The third test was conducted without the use of antimetabolite to

determine the degree of replication which occurs when the two instruments are tested simultaneously. Both responses were good, and the fact that one eventually became two times as great as the other is probably a reflection of the amount of inoculum.

The basal M8 medium with the radioactive formate, glucose, and lactate appears to be quite satisfactory for the soils tested.

FIELD TEST - GULLIVER III

Date: May 9, 1963

Weather: 27°C, sunny, slight wind

Location: Field - AMF

Orientation of Instrument: Upright

Ground Condition: Dry, dusty

Soil Sample Collected: Very good

Medium: 3 ml, M8, 6.16 uc/ml

Antimetabolite: 0.5 ml, Bard Parker

Radioactive Substrates:

(a) Formate - .66 mM Sp. Act 5.0 mc/mM, 3.30 μc/ml
(b) Glucose - .22 mM Sp. Act 3.0 mc/mM, .66 μc/ml
(c) Lactate - .44 mM Sp. Act 5.0 mc/mM, 2.20 μc/ml

Mechanical Function:

(a) Sequencing: No problems arose
(b) Projectiles: No problems arose
(c) Thermostat: Functioning

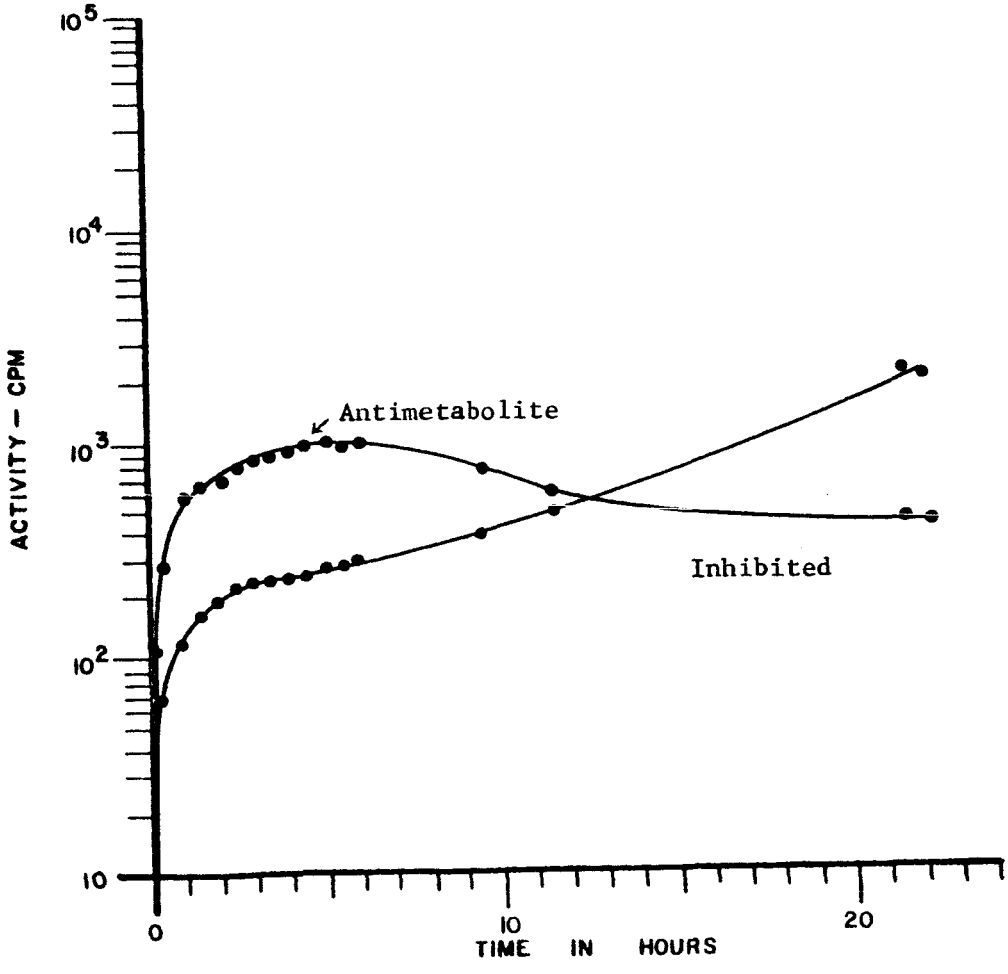
Components:

(a) Detector: Geiger Müller tube
(b) Collector: Gum coated, $Ba(OH)_2$

General Evaluation:

Mechanical operation was satisfactory. Low response may have been due to saturation of collector. Antimetabolite appeared to be effective. However, initial response from uninhibited test may be result of smaller inoculum.

Response:



FIELD TEST - GULLIVER III

Date: May 16, 1963 | Weather: 18°C, overcast, slight wind

Location: Field - AMF | Orientation of Instrument: Upright

Ground Condition: Dry, not very dusty | Soil Sample Collected: Good

Medium: 3 ml, M8, 6.16 uc/ml | Antimetabolite: 0.5 ml, Bard Parker

Radioactive Substrates:

(a) Formate - .66 mM Sp. Act 5.0 mc/mM, 3.30 μc/ml
(b) Glucose - .22 mM Sp. Act 3.0 mc/mM, .66 μc/ml
(c) Lactate - .44 mM Sp. Act 5.0 mc/mM, 2.20 μc/ml

Mechanical Function:

(a) Sequencing: No problems arose
(b) Projectiles: No problems arose
(c) Thermostat: Functioning

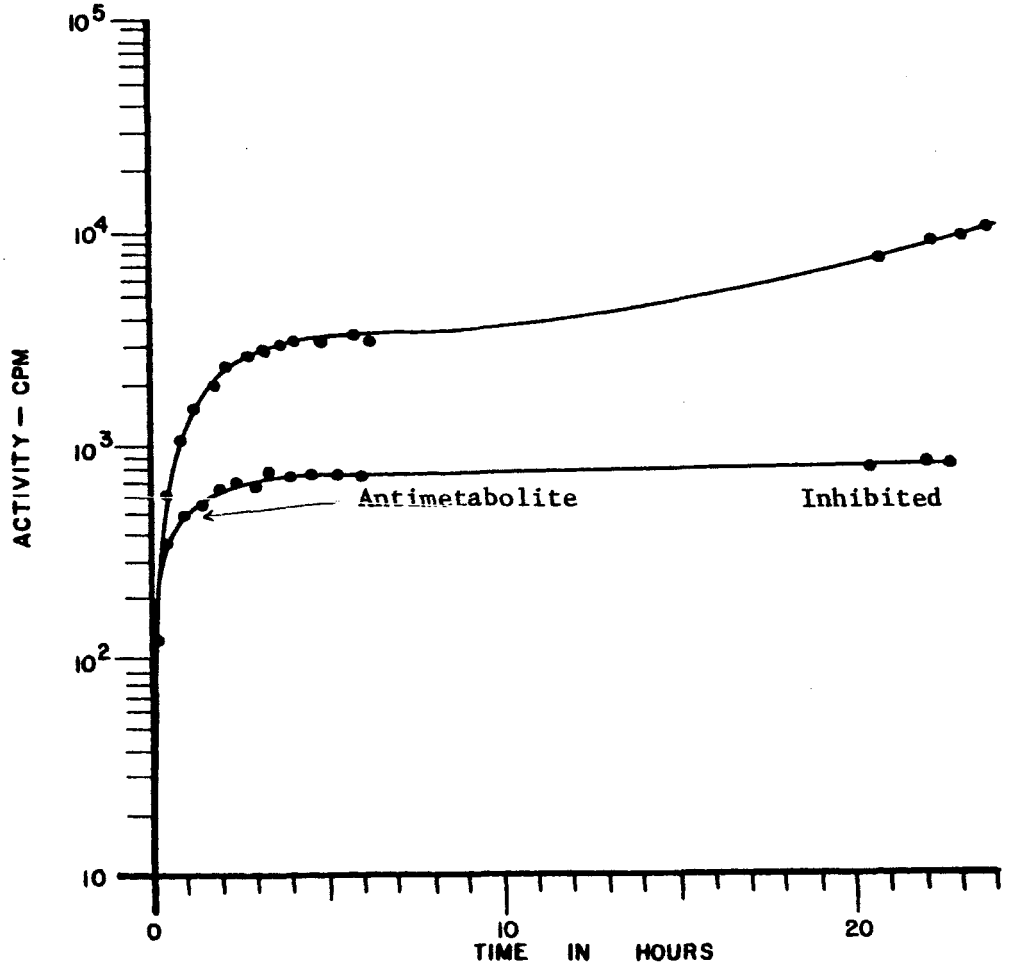
Components:

(a) Detector: Geiger Müller tube
(b) Collector: Gum coated, $Ba(OH)_2$

General Evaluation:

Satisfactory although collectors may have become saturated. Responses were moderate. The antimetabolite was effective.

Response:



FIELD TEST - GULLIVER III

Date: May 23, 1963

Weather: 13°C, Sunny, slight wind

Location: Field - AMF

Orientation of Instrument: Upright

Ground Condition: Hard

Soil Sample Collected: Good

Medium: 3 ml, M8, 6.16 uc/ml

Antimetabolite: None used

Radioactive Substrates:

(a) Formate - .66 mM Sp. Act 5.0 mc/mM, 3.30 μc/ml
(b) Glucose - .22 mM Sp. Act 3.0 mc/mM, .66 μc/ml
(c) Lactate - .44 mM Sp. Act 5.0 mc/mM, 2.20 μc/ml

Mechanical Function:

(a) Sequencing: No problems arose
(b) Projectiles: No problems arose
(c) Thermostat: Functioning

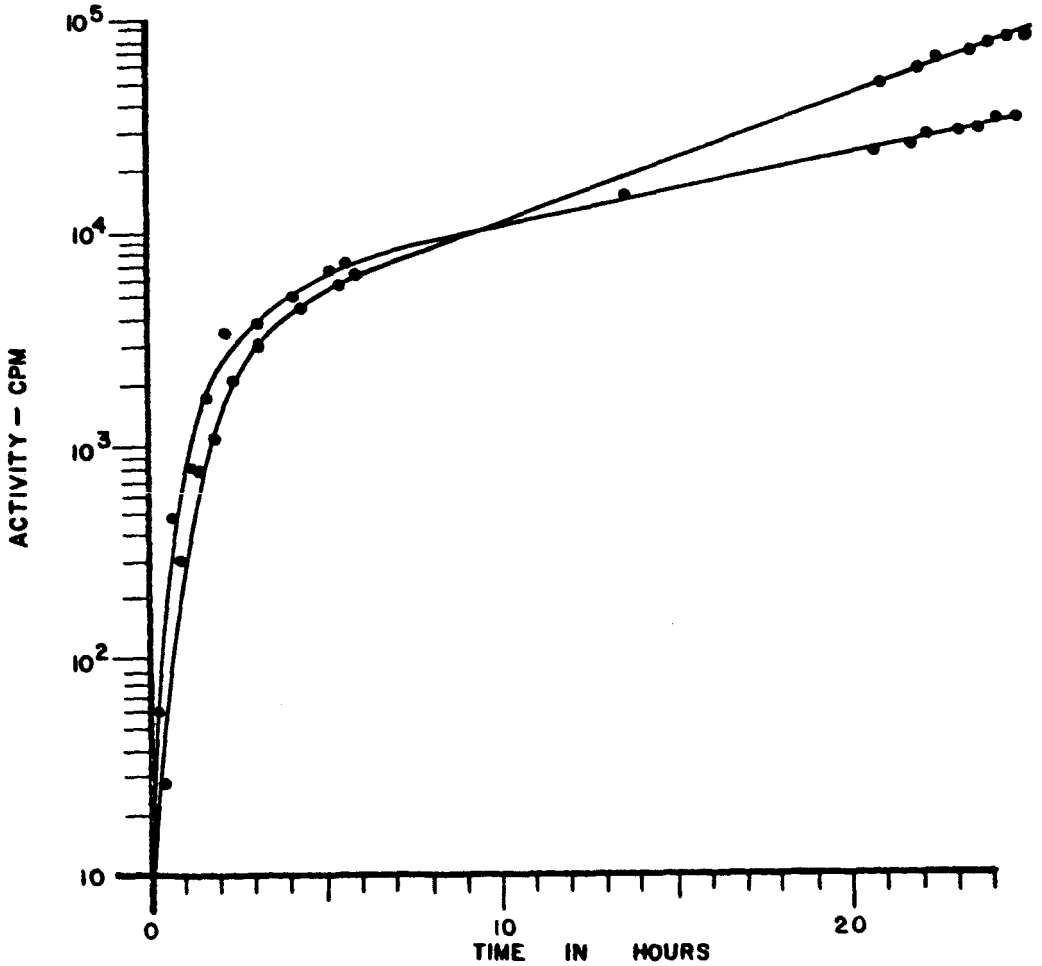
Components:

(a) Detector: Geiger Müller tube
(b) Collector: Krylon, $Ba(OH)_2$

General Evaluation:

Satisfactory. Antimetabolite was not used in either instrument. Both responses were good.

Response:



Quarterly Progress Report No. 9

NASr-10

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Respectfully submitted,

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III. INSTRUMENTATION

A. OBJECTIVES AND SCOPE

The two principal objectives of the instrumentation effort of the current program are to field test the existing Gulliver III instrument and to investigate, on a limited basis, ways to improve terrestrial field test response or sensitivity. Areas of improvement being investigated are beta radiation detectors, CO_2 gas collectors, soil sample collection, and nonmetabolic gas removal. Little or no efforts are to be expended for "hardening" or engineering components or equipment for the anticipated environments expected or for a vehicle for a Mars mission since the mission has not been assigned and the vehicle and mission program are not known.

Field tests are to be performed semiweekly with all but five to be in the greater Washington area. These numerous tests will permit field evaluations of various minor changes in the instrument and changes in the radioactive broth, and at the same time, instrument reliability and performance can be logged.

B. FIELD TESTING

The supply of expendable materials used for field testing was severely depleted at the completion of the last program; and before any field tests could be performed, supplies had to be replenished. This prevented performing more than three tests during this first reporting period, even though they were on a weekly instead of the specified semiweekly basis.

The instruments performed completely satisfactorily, mechanically and electronically, on all three tests. Problems were encountered with the CO_2 gas collectors, in that they became saturated. To check for saturation after a field test, the geiger tubes with $Ba(OH)_2$ on the window are exposed to $C^{14}O_2$ in a closed test chamber and changes in the counting rate are observed. In the first two tests, it was obviously a case of insufficient $Ba(OH)_2$. Tests are in progress to determine the possible causes of apparent saturation of the collector during the last test. Better controls are being instituted to prevent the gas collectors, after being deposited on the geiger windows, from collecting stable CO_2 from the atmosphere or from the assembler's exhaled breath before insertion and sealing in the instrument body.

C. RADIOISOTOPE DETECTION INVESTIGATIONS

1. Study of Detector Types

The state-of-the-art of various soft beta detectors is constantly being monitored in order to stay abreast of any new developments in the field that would improve the present system.

High on the list of detectors of interest are semiconductor detectors which were extensively investigated during the last program. These detectors offer several advantages over gas ionization devices in that they are very small, require low voltages, are solids and are therefore less susceptible to shock and vibration; and, due to their thin depletion regions, are inherently insensitive to particles that have low specific ionization characteristics -- such as cosmic

rays -- with consequent reduction of background interference. Two disadvantages are in stability and moderate sensitivity.

Much work is presently being done on these detectors, however, in other laboratories. AMF project personnel maintain contact with the leading manufacturers, researchers, and technical organizations that publish new developments in this field.

An Amperex gas-flow counter was purchased during the last program and is currently being used in investigations described below. Several different types of gas-flow counters have been ordered and tests will be conducted to compare various flow counters with each other as well as with geiger tubes.

2. Comparison Tests

A brief investigation was made at the end of the last program which exposed a thin-window geiger tube and a windowless gas-flow counter to a sealed volume of $C^{14}O_2$ and counting gas. Further tests were indicated in order to verify the initial results as well as to thoroughly investigate the effects due to water vapor and other gases evolved by the broth.

The first requirement was to ascertain that the Amperex gas-flow counter (Model 400 PC) was operating properly. The counter was connected in the circuit used previously, the pulses observed on an oscilloscope and a plateau obtained. The beta plateau slope was 7% per 100V from 1900V to 2100V with a dead time of about 300 microseconds. Furthermore, erratic behavior was observed on occasions.

Several steps were then taken to improve the performance. Circuit changes were made in the high voltage junction box. The anode wire was replaced with a finer wire (1.5 mil tungsten) which affects the gain, dead time and pulse height distribution. Occasional erratic behavior was eliminated by replacing the high voltage connector. Finally, the counter was cleaned with acetone, rinsed with isopropyl and baked at 70°C for two hours.

After repeating the plateau test, the results now showed a beta plateau slope of 3.5% per 100V from 1900V to 2200V. The dead time was reduced from 300 microseconds to about 6 microseconds. This improvement in performance was well worth the attention given the counter.

The next step was to install the flow counter in the test chamber that had been prepared for the comparison of geiger and windowless counters. After installation, several very thorough tests were performed to verify that the flow counter was operating properly under the new conditions, i.e., with its slide removed and its sensitive volume now exposed to the counting gas in the test chamber. A diagram of the apparatus to compare geiger and flow counters is shown in Figure 1. A small radium source was secured to the top of the counter before the latter was installed in the test chamber. Counts were then taken before and after installation on the chamber without moving the source in relation to the counter and with constant voltage and gain settings. Identical counts were obtained under the two conditions once the test chamber had been filled with the argon-methane counting gas.

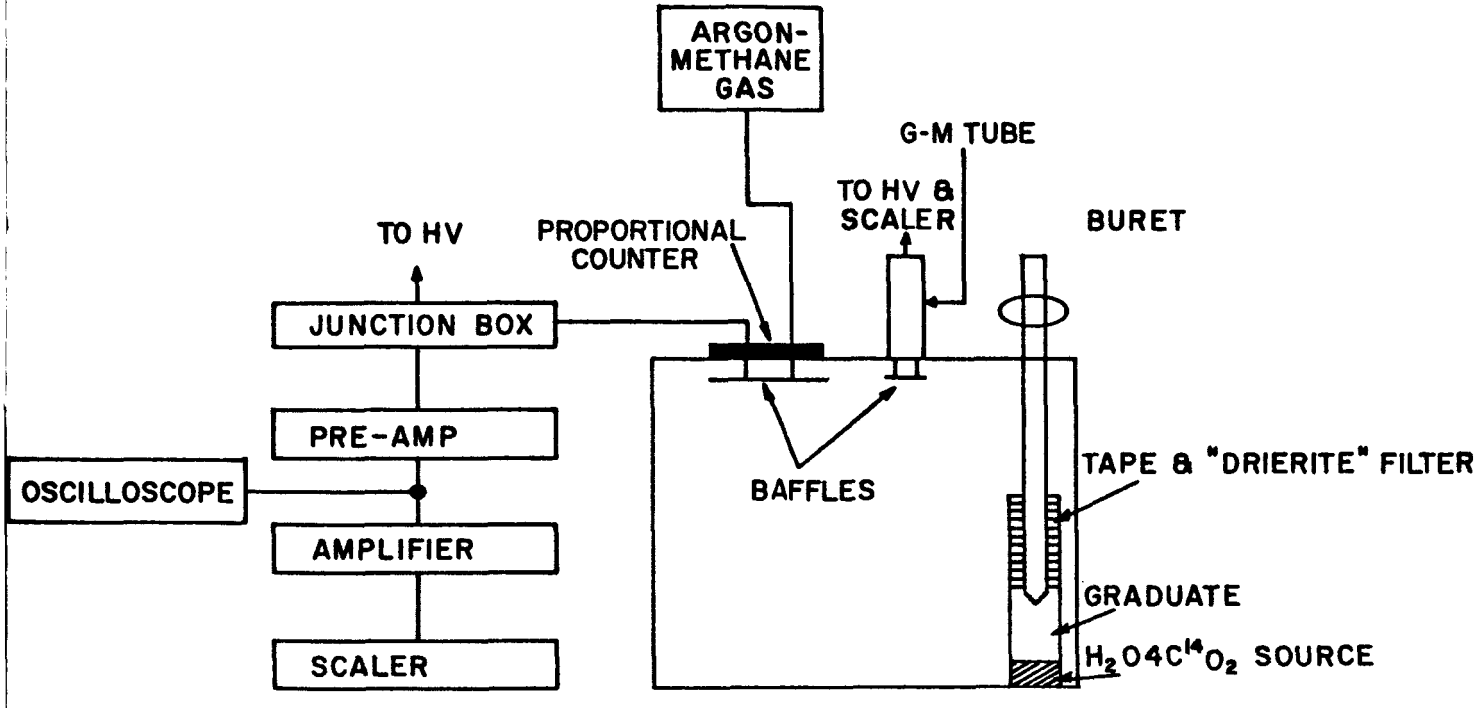

Figure III-1. Diagram of Apparatus for Comparing a Windowless Proportional Counter with a Thin End-Window Geiger Tube.

The next test was to ascertain the effect of water vapor on the counting. This was accomplished by introducing 2cc. of water into the test chamber via a buret. The effect was a serious degradation of the count rate in less than 20 minutes. This was not surprising as it is well known that water vapor is deleterious to many proportional counters.

Some technique is required to remove water vapor while allowing CO_2 to pass into the counter, since water vapor would be present above the broth in an incubation chamber and when CO_2 is generated in the test chamber by reaction of C^{14} tagged Na_2CO_3 solution on acetic acid crystals. Placing an open petri dish of Drierite (a commercial dessicant) in the test chamber as a second approach to this did not make much difference in the degradation due to the water problem. Pellets of Drierite were secured to approximately 4 inches of adhesive tape which was then wrapped around the bottom of the buret used to introduce water in the test chamber. The tape and buret were then inserted snugly, but not tightly, into a 25cc. graduate which is placed inside the chamber. The Drierite pellets serve not only to collect the vapor but also as spacers between layers of the tape. Thus, there are open paths for gases (H_2O and CO_2) liberated from the broth or carbonate-acid mixture to diffuse out of the graduate, but in doing so, they must come in contact with the Drierite which will absorb the H_2O but allow the CO_2 to continue its diffusion process. Utilizing this technique, 2cc. of water were introduced via the buret into the graduate and the effect on the counting observed. No degradation of the counting had occurred for one hour after the water was introduced at which time the test was concluded. Therefore, this technique appears to

offer a solution to the water vapor problem for purposes of these laboratory tests. More refined approaches would be required if H_2O removal were deemed necessary in Gulliver.

The final preliminary test was to insert a gas collector in the flow counter and determine if it had any degrading effects. The collector was constructed by applying $Ba(OH)_2$ to small pieces of Mylar previously cut to fit the inner surfaces of counter. The Mylar was then fitted in place with the hydroxide facing the counting volume and a screen grid placed over the Mylar. The grid served to hold the Mylar in place as well as to function as the new cathode since it was grounded. In this fashion, the walls of the counter were kept relatively clean and a conductive cathode surface was maintained. Repeating the counting tests performed previously showed that the counter was performing satisfactorily. Therefore, future tests with hydroxide on both the geiger window and inside the flow counter can be accomplished with assurance that the collector is not interfering with the results of interest.

At the time of writing this report, the first of the major tests comparing a windowless counter with a thin-window tube is ready to be undertaken. All of the above mentioned work was necessary as a preliminary to this most important test. The information obtained from this comparison will greatly determine the direction and amount of effort to be expended on adapting a windowless detector to Gulliver. If so indicated, tests with other gases, especially nitrogen at reduced pressure, will be performed in order to obtain a counting environment under anticipated Martian atmospheric conditions.

D. GAS COLLECTION, SAMPLE COLLECTION, AND NONMETABOLIC GAS REMOVAL INVESTIGATIONS

1. Gas Collection

Though the presently-developed method of spraying barium hydroxide powder onto a geiger tube face wet with krylon has proven satisfactory for field testing, there is a definite possibility that some other method may be developed to give better sensitivity and/or more absorptive capacity.

Krylon applied as above as an adhesive has the disadvantages of drying too fast to allow precise reproducible amounts of hydroxide to be applied or to be applied evenly. The cleaning of the tube is also rather difficult. In order to counteract these factors, a water-soluble gum has been under investigation as a possible adhesive. Since the gum drys slowly, more time is allowable to apply smooth, even coatings of hydroxide. In addition, the gum/hydroxide coating can be removed more easily.

At this experimental stage, no optimum gum/hydroxide coating has been found. Rather, the work has been directed toward developing a combination which adheres properly and which gives better performance than the krylon method used in the past.

The first experiment was to mix up a paint of gum and hydroxide and apply to a geiger tube for comparison to another tube coated by the krylon method. These two tubes were mounted in a test chamber and tagged CO_2 was released. Comparative count rates were taken as the tubes were thus exposed to the same CO_2 environment. The results of this were as follows:

Time after Start (Minutes)	Gum Coated (Total Count)	Krylon Coated (Total Count)
1	12,200	3,510
5	207,000	37,690
15	1,338.000	189,000
30	3,679,000	523,100

Though these data are not conclusive, they do indicate strongly that the gum technique merits further investigation. Consequently, more elaborate experimental apparatus was contrived so that multiple samples of varying types of coating could be exposed to the same tagged gas atmosphere and compared.

Essentially, the test chamber is a bowl with a coverplate. The coverplate has 12 holes to accommodate planchets or geiger tubes, and for introduction of tagged carbonate solution onto acid in the bottom of the bowl. A small battery-powered fan is mounted inside to assure circulation and thorough mixing of the evolving tagged CO_2.

The sampling planchets are modifications of standard 1 inch diameter planchets. These have been punched out so that a thin mylar window the size of the 18515 geiger tube can be cemented in place. When the window planchets are coated in the same manner as the geiger tubes, close simulation results. The exposed planchets can then be counted through the window via a geiger tube or in a gas proportional counter.

In the runs to date, the gum/hydroxide paint was prepared in a ball-mill and sprayed onto the planchet windows with an artist's airbrush. The conclusions so far are: (1) that the count rate of exposed planchets coated with the gum paint increases with the amount of paint applied; (2) no limit has been found

yet; (3) the count rate of krylon coated planchets also increases with the amount of hydroxide applied, but the proportional increase is not as great as with the gum paint; (4) the most effective amount of gum paint tried to date shows about 5 times higher count rate than the most effective krylon coating. All of these conclusions are based on counting the "windowed planchets" in a gas-flow counter.

2. Sample Collection

No work has been accomplished in this area during this quarter.

3. Nonmetabolic Gas Removal

Initial efforts in this area were curtailed in favor of the work on gas collection since reproducible, standardized collectors would enhance evaluation of the test data. No further conclusions beyond those of the last final report have been formulated.